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RETINAL DISORDERS

Predictive capacity of baseline hyperreflective dots on the intravitreal dexamethasone implant (Ozurdex®) outcomes in diabetic macular edema: a multicenter study

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Abstract

Purpose The purpose of this study is to evaluate the predictive capacity of the baseline hyperreflective dots (HRDs) on the functional and anatomical response in patients with diabetic macular edema (DME). Additionally, we assessed the impact of the intravitreal dexamethasone (DEX) implant on the functional and anatomic outcomes.

Methods Retrospective, multicenter study. The number of HRDs was graded in four different stages: [A] none HRDs; [B] few, 1–10 HRDs; [C] moderate, 11–20 HRDs; and [D] many, ≥21 HRDs. For statistical purposes, groups A and B were combined [scarce HRDs (S-HRDs)] and group D was renamed as [abundant HRDs (A-HRDs)]. The primary endpoints were the mean change in best corrected visual acuity (BCVA) and central macular thickness (CMT) according to baseline HRD stage.

Results One hundred eyes from one hundred patients were included in the study. Mean BCVA significantly improved from 52.9 (50.0 to 55.8) letters ETDRS at baseline to 57.2 (54.0 to 60.4) letters at month 6, $p = 0.0039$. There were no significant differences between the S-HRDs and A-HRD study groups in BCVA. As compared to baseline, CMT reduction was 106.3 (59.8 to 152.7) μm and 94.2 (34.7 to 153.7) μm in S-HRDs and A-HRD groups, respectively ($p < 0.0001$ each, respectively). Twenty-three (65.7%) and 18 (62.1%) eyes achieved a CMT reduction ≥ 10% in the S-HRD and A-HRD groups, respectively, $p = 0.7640$. DEX implant significantly reduced the presence of outer nuclear layer (ONL) disruptions ($p = 0.0010$).

Conclusions The number of HRDs did not influence either functional or anatomic outcomes. DEX implant significantly decreases the number of eyes with ONL disruptions, which might improve retinal integrity.

Keywords Diabetic macular edema · Hyperreflective dots · Dexamethasone · Ozurdex · Visual acuity · Central macular thickness

Introduction

Diabetic macular edema (DME) is a prevalent condition that represents a major cause of vision loss among working-aged individuals in developed countries [1, 2].

Although the incidence of vision loss among diabetic patients has decreased over the last several years, the number of cases suffering diabetic-related visual impairment has increased due to the greater burden of diabetic disease worldwide [3, 4].

Therefore, for healthcare systems, identification of optimal treatment strategies is crucial for providing patients a better long-term therapeutic management [5].

Despite DME is a major cause of visual loss, it is also among the most accessible to treatment. The main treatment options include intravitreal medical treatment (anti-VEGF or

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intravitreal corticosteroids therapies), laser photocoagulation and, eventually, surgical treatment by pars plana vitrectomy [6]. Since the introduction of intravitreal treatments, vascular endothelial growth factor (VEGF) inhibitors have become increasingly popular and now might be considered as the first-line treatment in DME, whereas intravitreal corticosteroids are indicated in cases with insufficient response or cases unsuitable for anti-VEGF therapies [6]. Interestingly, both strategies have demonstrated good functional and anatomic outcomes, even if they have different pathophysiological targets and affect different metabolic pathways, and both achieve better outcomes in treatment-naïve DME eyes [7–15]. Currently, we are not able to choose the best treatment option for an individual patient prior to treatment, because information about those baseline characteristics that might predict treatment outcomes has not been elucidated. If we could predict in advance the response to a specific treatment option, this would not only improve the clinical outcomes but also reduce the number of treatments and, therefore, the economic burden associated with the disease.

Assessment of the morphologic characteristics of DME may provide a better understanding of the pathophysiology of the disease, which in turn might help to select the best treatment option [16, 17].

Several spectral domain optical coherence tomography (SD-OCT) image biomarkers have been suggested in DME. One of these is the presence of hyperreflective dots (HRDs), which are well-circumscribed particles of 20–40 μm in diameter that have been associated with the presence of DME and have been frequently described in all retinal layers [18]. Their etiology has not been fully elucidated. Different hypotheses including extravasation of lipoprotein [19], migrating retinal pigment epithelium cells [20] or increased inflammation within the retina [21] have been proposed. The role of the HRDs in predicting clinical outcomes in patients undergoing treatment for macular edema has shown controversial results. While some studies have shown that the presence of HRDs was associated with poorer visual outcome in patients with macular edema [22, 23], Schreur et al. reported that a higher number of HRDs at baseline was associated with an adequate treatment response [24]. Interestingly, Hwang et al. observed that therapeutic response of certain treatments might be different according to the numbers of HRDs, being the hypothetical inflammatory origin of the HRDs a possible explanation of this finding [21, 23]. Certainly, there is increasing evidence that inflammatory processes play a considerable role in the DME pathogenesis [25–27]. Therefore, inhibiting inflammatory pathway mediators could be one of the therapeutic options for the DME. Ozurdex® (Allergan Inc., Irvine, CA, USA) is a bioerodible sustained release intravitreal implant that has been shown to be an effective treatment for DME in clinical and real-life studies [10, 11, 14, 15, 28].

The aim of this study is to evaluate the predictive capacity of the baseline HRDs on the functional and anatomical response in DME patients. Additionally, we assessed the impact of the intravitreal dexamethasone implant on the functional and anatomic outcomes, behavior of the HRDs, outer nuclear layer (ONL) disruptions, and external limiting membrane defects in a multicenter cohort of DME eyes.

Methods

Design

Retrospective, multicenter study conducted on the Ophthalmology Department of four tertiary hospitals in Spain (Hospital de Cruces, Bilbao; Hospital Clínic, Barcelona; Hospital Clínico San Carlos, Madrid and Hospital Universitario Vall de Hebrón, Barcelona). The study was carried out in accordance with the Helsinki Declaration, and institutional review board approval was obtained in Hospital de Cruces and Hospital Clínic of Barcelona as part of a non-interventional retrospective audit. Informed consent for study participation was waived because of the retrospective study design. All data concerning the patients' diagnosis and follow-up were extracted retrospectively from medical records.

Patients

The study was conducted on consecutive patients with diabetic macular edema, either naïve or previously treated, who underwent treatment with the intravitreal dexamethasone (DEX) implant Ozurdex® and had a minimum follow-up of 6 months.

Patients were excluded if they had macular edema due to any other condition, history of major ocular surgery (including cataract extraction, scleral buckle or any intraocular surgery) within the previous 6 months, history of laser 6 months prior to the DEX implant, active proliferative diabetic retinopathy, media opacities due to cataract or corneal disease, vitreous hemorrhage, and those whose SD-OCT images were of poor quality.

Study design and data collection

Data was collected at baseline and 1, 3, and 6 months after the implant injections.

Clinical data collected included demographic characteristics, DME duration; type and treatment of diabetes; current Hb1Ac and previous treatments for DME, BCVA (using ETDRS charts), and grade of diabetic retinopathy staging (according to International Clinical Diabetic Retinopathy Severity Scale [29]. SD-OCT images were captured using

two commercially available devices, 3D OCT-2000 Spectral Domain OCT (Topcon Medical Systems, Inc., Oakland, USA) and Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA, USA). Standard 6x6mm Macular Cubes centered at the fovea (512 × 128 A-scans) at baseline and 1 (± 1 week), 3 (± 2 weeks), and 6 (± 1 month) months after the implant injection were analyzed. DEX implant 0.7 mg was injected into the vitreous cavity using standard protocols [11]. After the DEX implant injection, retreatment was judged necessary according to the physician criterion. It was mainly based on an anatomical criterion (central macular thickness (CMT), > 250 μm).

SD-OCT image analysis

SD-OCT images were quantitatively and qualitatively analyzed. Regarding the quantitative analysis, the macular thickness (CMT) in all the ETDRS areas (central retinal thickening—ETDRS area 9—and the other 8 ETDRS areas) was collected. Qualitative analysis included DME type according to Otani et al. [30] description, the number of HRD and the presence of abnormalities in the ONL, external limiting membrane (ELM) or ellipsoid zone. HRDs were defined as small, round- or oval-shaped, well-circumscribed particles (no bigger than 40 μm in diameter), with higher reflectivity than the background [18]. The number of HRDs in the central 25 horizontal scans of the 6 × 6 mm Macular Cube covering an area of 3000-μm radius centered on the fovea was manually counted by blinded evaluation, by a retina specialist, in all individual centers (AF, EB, AV, SP). In each individual, the number of HRDs was manually counted and subjectively graded according to the grading system previously reported by Framme et al. [19, 20]. According to this, the number of HRDs was graded in four different stages: [A] none HRDs; [B] few, from 1 to 10; [C] moderate, from 11 to 20 HDRs; and [D] many, 21 or more HRDs [19, 20]. In order to mitigate as much as possible the limitations and bias associated with the subjective evaluation of the number of HRDs, finally only two groups were considered for analysis: scarce HRDs (10 or less dots) and abundant HRDs (21 or more dots). Abnormalities in the ONL and ELM were also defined as any disruptions in these structures located subfoveally.

Outcome measures

The primary endpoints were the mean change between baseline and last follow-up visit in BCVA and CMT according to baseline HRDs stage. Secondary endpoints included percentage of patients gaining ≥ 5, ≥ 10, and ≥ 15 letters in BCVA according to the baseline HRDs stage; proportion of patients achieving an adequate anatomical response (CMT decrease ≥ 10%); HRD behavior; DME type, ellipsoidal zone disruptions; ONL disruptions; external limiting membrane defects; and retinal thickness in the different measured areas between the baseline and month 6 visit.

Statistical analysis

A standard statistical analysis was performed using the MedCalc Statistical Software version 18.11 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018).

Data are expressed as number (percentage), mean [standard deviation (SD)], mean [95% confidence interval (95% CI)], mean [standard error (SE)], or median (95% CI) as appropriate.

Data were tested for normal distribution using a D'Agostino-Pearson test.

If data were normally distributed, a repeated measure analysis of variance (ANOVA) and the Greenhouse–Geisser correction was used for the determination of the changes in BCVA and in macular thickness, between the baseline and each visit, into the groups. We used a linear mixed model in order to consider the correlations between the repeated measures and the existence of missing data. If data were no normally distributed, the comparisons of the changes in visual acuity and in macular thickness were performed using a Friedman's two-way analysis test.

The Mann–Whitney *U* test was used in the evaluation of the baseline continue variables between the different HRDs stages.

The chi-squared test was used to analyze the differences in HRDs stage, type of DME, ellipsoidal zone disruptions, ONL disruptions, and external limiting membrane defects between the baseline and month 6 visit.

Categorical variables were compared using a chi-square test and a Fisher's exact test, as appropriated.

The significance of differences in mean change BCVA and CMT between groups was analyzed using analysis of covariance (ANCOVA). The model included baseline "HRD stage" as a factor and baseline DME subtype, diabetic retinopathy staging, and study center as covariates.

Results

Baseline characteristics

Among the 122 eyes from 122 patients selected, 100 eyes from 100 patients fulfilled the inclusion/exclusion criteria and were included in the study. The main demographic and clinical characteristics have been summarized in Table 1.

Mean (95% CI) age in the overall study population was 67.5 (65.5 to 69.5) years. Sixty-eight (68.0%) eyes had nonproliferative DR.

Table 1 Baseline demographic and clinical characteristics in the scarce hyperreflective dot (S-HRD) and abundant hyperreflective dot (A-HRD) study groups

	Scarce HRDs[1], $N = 35$	Abundant HRDs[2], $N = 29$	p[a]
Age (years)			
Mean (SD)	69.4 (10.9)	66.7 (9.9)	0.2299
95% CI	65.7 to 73.1	62.9 to 70.4	
Sex, *n* (%)			
Male	22 (62.9)	21 (72.4)	0.4389[c]
Female	13 (37.1)	8 (27.6)	
HB1A (%)			
Mean (SD)	7.7 (1.4)	7.3 (0.9)	0.4274
95% CI	7.2 to 8.2	6.9 to 7.6	
Type of diabetes, *n* (%)			
Type 1	2 (5.7)	0 (0.0)	0.4965[c]
Type 2	33 (94.3)	29 (100.0)	
Type of DME, *n* (%)			
DRT	5 (14.3)	6 (21.4)	0.3373[b]
CME	25 (71.4)	15 (53.6)	
SRD	5 (14.3)	7 (25.0)	
Length of diabetes (months)			
Mean (SD)	244.9 (135.6)	194.6 (109.6)	0.1017
95% CI	197.6 to 292.2	152.1 to 237.1	
Diabetes treatment, *n* (%)			
Oral antidiabetics	6 (17.1)	11 (39.3)	0.1032[b]
Insulin	10 (28.6)	8 (28.6)	
Both	19 (54.3)	9 (32.1)	
Degree of DR, *n* (%)			
Mild	4 (11.4)	1 (3.4)	0.4502[b]
Moderate	9 (25.7)	12 (41.4)	
Severe	8 (22.9)	6 (20.7)	
Proliferative	14 (40.0)	10 (34.5)	
HBP, *n* (%)			
Yes	28 (80.0)	18 (62.1)	0.1632[c]
No	7 (20.0)	11 (37.9)	
BCVA, letters*			
Mean (SD)	53.2 (13.1)	51.6 (16.2)	0.3042
95% CI	48.7 to 57.7	45.4 to 57.8	
CMT (μm)			
Mean (SD)	482.0 (138.1)	492.1 (151.1)	0.9301
95% CI	434.6 to 529.4	434.7 to 549.6	
Previous DME treatments, *n* (%)			
Naïve	5 (14.3)	4 (13.8)	0.1414[b]
Laser (focal)	6 (17.1)	1 (3.4)	
Anti-VEGF	3 (8.6)	8 (27.6)	
Steroids	0 (0.0)	0 (0.0)	
Surgery	1 (2.9)	0 (0.0)	
Combination**	20 (57.1)	16 (55.2)	
EZ disruptions, *n* (%)			
Yes	22 (62.9)	21 (75.0)	0.4155[c]
No	13 (37.1)	7 (25.0)	
ONL disruptions, *n* (%)			
Yes	30 (85.7)	28 (100.0)	0.0602[c]
No	5 (14.3)	0 (0.0)	
ELM defects, *n* (%)			
Yes	25 (71.4)	17 (60.7)	0.4270[c]
No	10 (28.6)	11 (39.3)	

S-HRDs scarce hyperreflective dots, *A-HRDs* abundant hyperreflective dots, *N* number, *SD* standard deviation, *CI* confidence interval, *DME* diabetic macular edema, *DRT* diffuse retinal thickness, *CME* cystoid macular edema, *SRD* serous retinal detachment, *DR* diabetic retinopathy, *HBP* high blood pressure, *BCVA* best-corrected visual acuity, *CMT* central macular thickness, *VEGF* vascular endothelial grow factor, *EZ* ellipsoidal zone, *OPL* outer nuclear layer, *ELM* external limiting membrane

[a] Mann–Whitney *U* test (between early-switch and late-switch eyes)

[b] Chi-square test

[c] Fisher's exact test

*Letters in the Early Treatment Diabetic Retinopathy Study (ETDRS) charts

**Combination of some of the above

[1] Number of HRDs ranged from 0 to 10

[2] Number of HRDs > 20

Regarding the HRDs, at baseline, 4 eyes had none HRDs, 31 eyes had 1–10 HRDs, 36 eyes had 11–20 HRDs, and 29 eyes had ≥21 HRDs.

Forty-one eyes received an additional DEX implant during the study follow-up, in all the cases between months 4 and 5 of follow-up.

Visual outcomes and HRDs

Mean BCVA significantly improved from 52.9 (50.0 to 55.8) letters ETDRS at baseline to 59.5 (56.8 to 62.2); 60.7 (57.6 to 63.7) and 57.2 (54.0 to 60.4) letters at 1, 3, and 6 months of follow-up, respectively; $p < 0.0001$, $p < 0.0001$, and $p = 0.0039$, respectively.

There were no significant differences between the S-HRD and A-HRD study groups in BCVA over the course of follow-up (Fig. 1). The mean (standard error of the mean, SEM) change, from baseline to month 6, in BCVA was similar in both groups [3.4 (3.5) letters, $p = 0.3361$, ANCOVA with Bonferroni correction]. Nevertheless, the BCVA significantly improved, in both groups, as compared to baseline ($p < 0.05$ each, respectively; repeated measures ANOVA and the Greenhouse–Geisser correction).

At month 6, in the overall study population, the proportion of patients gaining ≥ 5 letters, ≥ 10 letters, and ≥ 15 letters was 52.0%, 33.0%, and 22.0%, respectively. There were no significant differences between groups (Table 2).

CMT and HRDs

In the overall study population, CMT was significantly reduced from 495.1 (468.9 to 521.3) μm at baseline to 333.5 (314.4 to 352.6) μm, 358.3 (332.2 to 384.3) μm, and 390.1 (361.5 to 418.7) μm at 1, 3, and 6 months of follow-up, respectively; $p < 0.0001$ each, respectively. Sixty-eight (68%) eyes obtained a CMT reduction ≥ 10% from baseline.

As compared to baseline, CMT reduction was 106.3 (59.8 to 152.7) μm and 94.2 (34.7 to 153.7) μm in S-HRD and A-HRD groups, respectively ($p < 0.0001$ each, respectively).

As with the BCVA, there were no significant differences between the S-HRD and A-HRD study groups in CMT over the course of follow-up (Fig. 2).

Twenty-three (65.7%) eyes in the S-HRDS group and 18 (62.1) eyes in the A-HRD group achieved a CMT reduction ≥ 10% ($p = 0.7640$, chi-squared test).

Table 3 shows the mean change, between baseline and month 6, of the different SD-OCT measured areas for study groups.

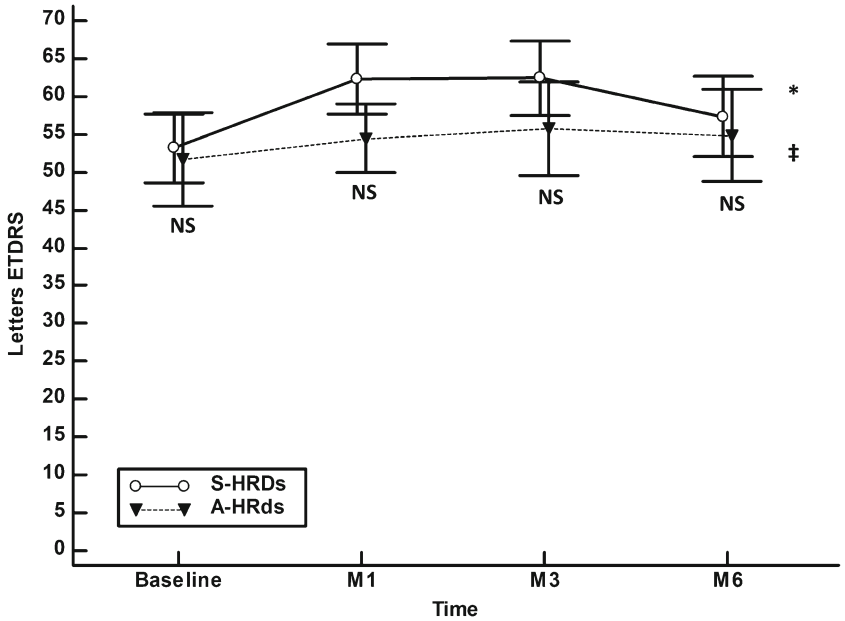

Fig. 1 Mean best-corrected visual acuity (BCVA) over the course of follow-up in the scarce hyperreflective dots (S-HRDs) and abundant hyperreflective dots (A-HRDs) study groups. The vertical bars represent the 95% confidence interval. There were no statistically significant differences between groups at any of the different time points. Statistical significance, at the different time point measurements, was determined using the analysis of covariance (ANCOVA). The model included baseline "HRDs stage" as a factor and baseline DME subtype, diabetic retinopathy staging and study center as covariates. Statistical significance, between baseline and month 6, was determined using repeated measures ANOVA and the Greenhouse–Geisser correction. NS not significant. Mean BCVA was significantly improved from baseline to month 6 in 4.1 (0.3 to 7.9) and 4.4 (1.3 to 7.5) letters in the S-HRD and A-HRD groups, respectively. *$p < 0.05$ as compared to baseline in S-HRD group. ‡$p < 0.05$ as compared to baseline in A-HRD group

Table 2 Proportion of patients achieving a best-corrected visual acuity (BCVA) improvement ≥ 5, ≥ 10, and ≥ 15 letters according to the baseline hyperreflective dot (HRD) stage

BCVA improvement	Scarce HRDs[1], $N = 35$	Abundant HRDs[2], $N = 29$	p[a]
≥ 5 letters*			
No	17 (48.6)	16 (55.2)	0.6018
Yes	18 (51.4)	13 (44.8)	
≥ 10 letters*			
No	21 (60.0)	22 (75.9)	0.1819
Yes	14 (40.0)	7 (24.1)	
≥ 15 letters*			
No	25 (71.4)	24 (82.8)	0.3724
Yes	10 (28.6)	5 (17.2)	

BCVA best-corrected visual acuity, *HRDs* hyperreflective dots

*Letters in the Early Treatment Diabetic Retinopathy Study (ETDRS) charts

[a] Chi-square test

[1] Number of HRDs ranged from 0 to 10

[2] Number of HRDs > 20

Behavior of HRDs, type of DME, and outer retina abnormalities

Table 4 summarizes the behavior of HRDs, type of DME, ellipsoidal zone disruptions, ONL disruptions, and external limiting membrane defects over the course of follow-up. A significant reduction in the frequency of ONL disruption was observed throughout follow-up ($p = 0.0010$).

Although was not statistically significant, 12 (20.0%) and 10 (17.2%), respectively, changed their ELM and EZ status from "disruption" to "normal" from baseline to the month 6 visit.

Outer retina abnormalities and visual outcomes

The eyes that changed their ONL status from "ONL disruption" at baseline to "Normal ONL" at month 6 [15 (17.6%) eyes) had a BCVA improvement of 12.9 (11.9) letters, which was significantly greater than in those eyes that remained as "ONL disruption" [1.7 (11.5) letters], $p < 0.0001$. The mean change in BCVA was significantly greater [12.7 (6.3) letters] in those eyes without ONL disruptions at baseline as compared with those with ONL disruption at baseline [4.0 (12.7) letters], $p = 0.046$. Moreover, such difference was even greater when considering those eyes without ONL disruptions at month 6 [12.8 (10.9) vs 1.8 (11.5) letters, respectively, $p < 0.0001$].

Regarding ELM, although the number of eyes that changed their ELM status was not statistically significant, among the 60 eyes with ELM disruption at baseline, 12 (20%) eyes

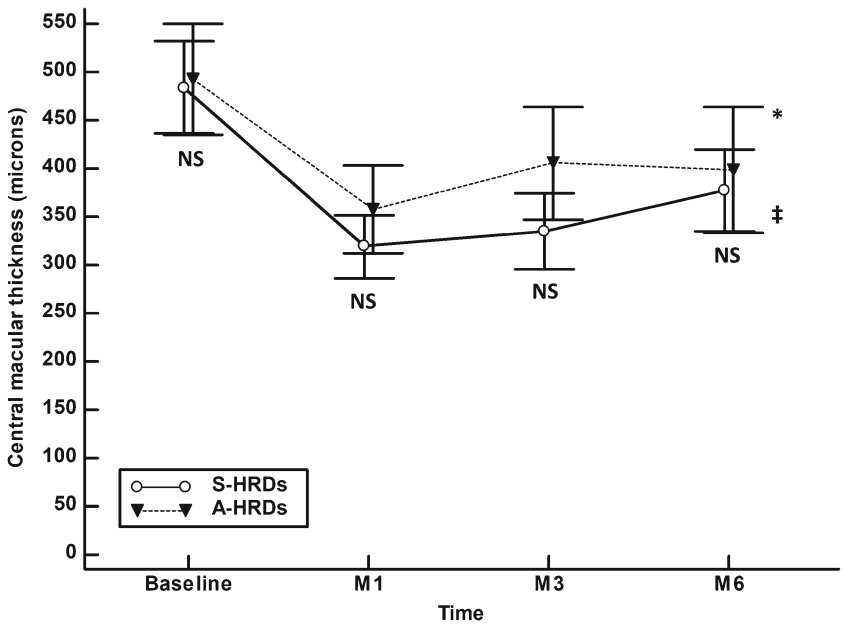

Fig. 2 Mean central macular thickness (CMT) over the course of follow-up in the scarce hyperreflective dot (S-HRD) and abundant hyperreflective dot (A-HRD) study groups. The vertical bars represent the 95% confidence interval. There were no statistically significant differences between groups at any of the different time points. Statistical significance, at the different time point measurements, was determined using the analysis of covariance (ANCOVA). The model included baseline "HRDs stage" as a factor and baseline DME subtype, diabetic retinopathy staging, and study center as covariates. Statistical significance, between baseline and month 6, was determined using repeated measures ANOVA and the Greenhouse–Geisser correction. NS not significant. Mean BCVA was significantly improved from baseline to month 6 in 4.1 (0.3 to 7.9) and 4.4 (1.3 to 7.5) letters in the S-HRD and A-HRD groups, respectively. *$p < 0.0001$ as compared to baseline in S-HRD group. ‡$p < 0.0001$ as compared to baseline in A-HRD group

Table 3 Overview of the values of spectral domain optical coherence tomography (SD-OCT) measured areas and their changes from baseline for study groups

	S-HRDs[1]		A-HRDs[2]		Difference between treatment groups	
Variable	Mean (95% CI) from baseline	p^a	Mean (95% CI) from baseline	p^a	Mean (95% CI)	p^b
Area 1 (μm)	− 19.2 (− 37.1 to − 1.4)	0.0355	− 23.0 (− 68.4 to 22.4)	0.3080	− 3.7 (− 48.1 to 40.7)	0.4397
Area 2 (μm)	− 20.4 (− 36.8 to − 4.0)	0.0165	− 48.6 (− 93.2 to − 4.0)	0.0338	− 28.2 (− 72.2 to 15.8)	0.2270
Area 3 (μm)	− 25.1 (− 50.0 to − 0.3)	0.0479	− 32.9 (− 83.8 to 18.1)	0.1962	− 7.8 (− 60.9 to 45.4)	0.3261
Area 4 (μm)	− 14.8 (− 32.0 to 2.4)	0.0893	− 28.2 (− 70.6 to 14.2)	0.1865	− 13.5 (− 56.0 to 13.1)	0.2963
Area 5 (μm)	− 53.2 (− 76.8 to − 29.7)	0.0001	− 34.2 (− 72.0 to 3.6)	0.0742	20.4 (− 22.1 to 63.0)	0.2414
Area 6 (μm)	− 51.2 (− 81.5 to − 21.60)	0.0016	− 51.6 (− 101.5 to − 1.7)	0.0431	− 0.4 (− 55.2 to 54.5)	0.6397
Area 7 (μm)	− 61.8 (− 87.8 to − 35.9)	< 0.0001	− 42.2 (− 86.2 to 1.9)	0.0597	19.7 (− 28.4 to 67.8)	0.5032
Area 8 (μm)	− 58.3 (− 81.7 to − 34.9)	< 0.0001	− 21.6 (− 70.8 to 27.5)	0.3740	36.7 (− 14.3 to 87.6)	0.2211

[a] Repeated measures ANOVA and the Greenhouse-Geisser correction

[b] Mann–Whitney *U* test

[1] Number of HRDs ranged from 0 to 10

[2] Number of HRDs > 20

S-HRDs scarce hyperreflective dots, *A-HRDs* abundant hyperreflective dots, *CI* confidence interval

Table 4 Overview of the behavior of the hyperreflective dots (HRDs), type of diabetic macular edema (DME), ellipsoidal zone (EZ) disruptions, outer nuclear layer (ONL) disruptions, and external limiting membrane (ELM) defects throughout the study in the overall study population. *p*-values were calculated by using the chi-squared test

Variable	Baseline	Month 1	Month 3	Month 6	*p*
HRDs, *n*					
None	4	10	5	8	0.1746
1–10	31	31	34	36	
11–20	26	31	38	20	
≥ 21	29	25	17	25	
Type DME, *n*					
No edema	0	13	13	8	0.0025*
DRT	15	23	21	23	
CME	59	44	46	48	
SRD	20	10	7	10	
EZ disruptions, *n*					
Yes	58	47	43	49	0.2779
No	36	46	46	41	
ONL disruptions, *n*					
Yes	85	67	59	68	0.0010
No	9	26	30	22	
ELM defects, *n*					
Yes	60	47	45	50	0.2189
No	34	46	44	40	

HRDs hyperreflective dots, *n* number of eyes, *DME* diabetic macular edema, *DRT* diffuse retinal thickness, *CME* cystoid macular edema, *SRD* serous retinal detachment, *EZ* ellipsoidal zone, *ONL* outer nuclear layer, *ELM* external limiting membrane

*If we do not take into account the cases of edema resolution, the *p* value was 0.1502

changed their ELM status to "ELM normal" at month 6. In these eyes, the mean BCVA improvement was significantly greater [12.8 (11.6) letters] than in those that did not change their ELM status [2.4 (12.4) letters], $p < 0.0001$.

However, although the BCVA improvement was greater in the 10 (17.2%) eyes that changed their status, between baseline and month 6, from "EZ disruption" to "EZ normal" [10.7 (12.8) letters] than in those that did not change [2.8 (12.7)], such a difference was not statistically significant ($p = 0.0547$).

Discussion

The results of the current study suggested that, independently of the number of HRDs at baseline, DEX implant achieved a significant functional and anatomical improvement in patients with DME.

Our study did not find significant differences in either BCVA improvement or CMT reduction between those eyes with few HRDs and those with many HRDs.

These findings differ from those papers suggesting that higher number of HRDs were associated with poorer functional and anatomic outcomes [22, 23], as well as from those suggesting the opposite one: higher number of HRDs were associated with better outcomes [21, 24, 31].

However, it is not easy to compare our results with those of the aforementioned studies [21–24, 31]. Chatziralli et al. [22], Schreur et al. [24], and Kang et al. [31] evaluated the effect of anti-VEGF. Additionally, Hwang et al. [23] evaluated patients with DME or macular edema due to retinal vein occlusion.

Such differences may be due to substantial differences in study designs, study population, and different criteria for treatment response.

An interesting point is the fact that in our study higher baseline number of HRDs was not associated with poorer baseline BCVA. This finding differs from that reported by Schreur et al. [24] and Uji et al. [32], who found an association between higher baseline number of HRDs and poorer baseline BCVA. Uji et al. reported that the presence of HRDs in the outer retina was closely associated with a disrupted ELM and in the inner and outer segment line [32]. However, our study found that, at baseline, there were no differences in either EZ disruptions; ONL disruptions or ELM defects between those eyes with scarce number of HRDs and those with abundant number of HRDs.

Interestingly, Hwang et al. [23], who reported a significative number of HRDs at baseline in bevacizumab non-responders, found that eyes that responded to DEX implants had a significantly greater number of HRDs than those eyes that did not respond to DEX implants. Moreover, Vujosevic et al. [21] found that in eyes treated with DEX implant, higher number of HRDs at baseline was correlated with higher increase in retinal sensitivity. Whereas in eyes treated with VEGF inhibitors, the number of HRDs correlated inversely with retinal sensitivity [33]. This suggested that different patient profiles might have different therapeutic responsiveness [21, 23, 33].

The origin of the HRDs remains unclear. Various different hypotheses have been proposed about the origin of HRDs that might be non-exclusive indeed. Bolz et al. [34], who first described such hyperreflective dots, suggested that HRDs are the morphological manifestations of lipid extravasation in DME. However, other authors have developed other hypotheses, for what causes the HRDS: extravasation of lipoprotein [19], migrating retinal pigment epithelium cells [20], or an increased inflammation in the retina [21].

It has been reported a significant association between the soluble form of the cytokine CD14 and the presence of HRDs in the inner retina, suggesting an activation of the microglia and severe inflammation in DME patients [35].

Current advances in research led to significant improvements in understanding DME specific pathogenic mechanisms; there is increasing evidence that inflammatory processes have a considerable role in the pathogenesis of diabetic retinopathy (DR) and DME [25–27]. Therefore, inhibiting inflammatory pathway mediators could be one of the therapeutic options for the DME.

On the other hand, it has been suggested that HRDs amount might be an indicator of disease severity. Fewer HRDs might reflect better tissue integrity whereas the presence of many HRDs reflects tissue disintegrity representing more severe DME conditions [32].

We did not observe an effect of DEX implant on the number of HRD over the course of follow-up. An effect on EZ and ELM was neither observed. Nevertheless, the number of eyes with ONL disruptions decreased, an effect that might be attributed to DEX implant.

We do not have a definitive pathophysiological explanation that explained the statistically significantly positive effect on the ONL, but no effects on the EZ or ELM, though this finding might be related with the study sample. It might be hypothesized that, although it would seem that a similar behavior could be expected for the three elements (ONL, EZ, and ELM) since their close localization in the outer retina, it should be highlighted that they have completely different histologic structures; hence, it might be speculated that dexamethasone may exert a specific beneficial effect for the ONL, that is for the nucleus area of the photoreceptors, but not for their mitochondria (EZ) or for the junctions between them and the Müller cells (ELM).

The recovery of ONL integrity was associated with better functional outcomes in our patients, a plausible and expected observation due to the key role of this structure in visual function.

The results showing a significant improvement in BCVA with DEX implant did not significantly differ from those previously published [9–15, 28].

Additionally, 68 (68.0%) eyes achieved a CMT reduction $\geq 10\%$ as compared to baseline. Finally, most of the retinal areas achieved a significant thickness reduction as compare to baseline, with none of the areas showing significant differences in thickness reduction between S-HRD and A-HRD study groups.

This study has some limitation that should be taken into consideration when interpreting the results. The first limitation is its retrospective design. Confounding factors and bias are inherent to retrospective studies. Nevertheless, this study selected strict inclusion/exclusion criteria for minimizing that issue. Another limitation is the system to classify HRDs. There could be certain bias with our system. Nevertheless, in order to reduce the bias, the group C (HRDs from 11 to 20) was not included in the grouped analysis. An additional limitation is the lack of intraobserver and interobserver reproducibility or repeatability studies before to start the study. Nevertheless, reproducibility of OCT has been demonstrated [36].

Despite these limitations, the results of our study suggested that the number of HRDs did not influence either functional or anatomic outcomes of the DEX implant Ozurdex®. Interestingly, this study found that DEX implant was capable to significantly decrease the number of eyes with ONL disruptions, which might improve retinal integrity. Further studies are needed to identify predictors of DME therapies and for individualizing therapeutic strategies according to patients' characteristics.

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Compliance with ethical standards

Conflict of interest Drs. Alejandro Fonollosa and Javier Zarranz-Ventura have received a grant from Allergan during the conduct of the study.

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Research involving human participants and/or animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent The local ethics committee waived the need for written informed consent of the participants.

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